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Cytogenetic and Teratogenic Test of Polybrominated Biphenyls in Rodents

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Previously we reported negative terata and c-mitosis synergism of FireMaster (polybrominated biphenyls) with colchicine in subacutely treated rats. Now we report absence of chromosome aberrations *from FireMaster and absence of c-mitosis synergism of FireMaster and colchicine in male mice.*

For the study of chromosome aberrations groups of three mice received 0, 50, or 500 mg/kg FireMaster or 4.5 mg/kg triethylenemelamine (TEM) dissolved in dimethyl sulfoxide through a single stomach gavage administration. Five hours before killing the animals were injected with 5 mg colchicine/kg. Groups of 5 mice from each treatment were killed 12, 24, and 48 hr after treatment. From the bone marrow of each of 36 mice 100 metaphases were scored for gaps, chromatid and chromosome breaks, rearrangements and pulverized chromosomes. Only TEM induced chromosome damage.

For detection of synergism between FireMaster and colchicine, slides prepared for chromosome analysis were also scored for metaphase and mitotic indeces. Control mice for detection of synergism were treated as for the chromosome study but were not injected with colchicine. Approximately 1000 cells were scored from each of 72 animals for determination of metaphase and mitotic indeces. FireMaster did not show c-mitosis synergism with colchicine in mice.

Treatment with FireMaster did not cause visually recognizable toxicity.

Introduction

Through accidental contamination of animal feed with polybrominated biphenyls (PBB) several hundred Michigan farm families were contaminated with PBB from animal food products (*1*). Toxic and health data on PBB is scarce since PBB was not meant for animal or human consumption.

Toxic polychlorinated biphenyls (PCBs) (*2, 3*) are chemically similar to PBBs. Biological similarity of PCBs and PBBs is indicated by similar concentrations excreted in milk fat of cows (*4*). PCBs had no effect on mitotic division or chromosomal aberrations in rat bone marrow (*5*). Previously we reported results with 15 pregnant rats force fed with 100 mg PBB/kg in 1 ml corn oil six times in 2-day intervals beginning on the day 6 of pregnancy (*6*). Fourteen control animals received only corn oil. On the day 19 of pregnancy the animals were killed, and 376 fetuses were weighed, sexed, visually observed for cleft palates, missing digits, and gross malformations, and were stained with Alizarin Red S for skeletal abnormalities. Dead implants were also counted. For all parameters no difference was found between control and treated groups. There was also no difference in the weight gain and general health of treated and control animals, but a synergism of PBB with colchicine for mitotic arrest was observed in the bone marrow of pregnant rats (*6*).

This report demonstrates the absence of chromosome aberrations or mitotic arrest in bone marrow of male mice treated with PBB. The positive control triethylenemelamine (TEM) induced a high frequency of chromosome damage.

Materials and Methods

Random bred Upjohn strain Swiss albino male mice weighing 35± 2 g were used. The test sample used in the present and previous investigation (*6*)

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was from a bag of FireMaster (PBB) found in the grain mill where the contamination occurred and was acquired from Dr. R. Ringer of the Department Poultry Science of Michigan State University. TEM was from Pfaltz and Bayer Inc.

Cytogenetic Analysis of Bone Marrow

For the study of chromosome aberrations, groups of nine mice received 0, 50, or 500 mg FireMaster or 4.5 mg TEM/kg dissolved in 0.3 ml dimethyl sulfoxide (DMSO) through a single stomach gavage administration. After extensive trial and error runs with alcohols and various other organic solvents, PBB was found to be most soluble in DMSO. The animals were injected with 5 mg colchicine/kg 5 hr before killing. Groups of three mice from each treatment were killed 12, 24, and 48 hr after treatment.

Bone marrow was flushed through each end of the excised femurs with 1 ml of 2.5% sodium citrate from a syringe with a 23G needle. The bone marrow cells were suspended and were collected in a Clay Adams Dynac Centrifuge at 1000 rpm for 5 min. The supernatant was poured off, leaving about 0.25 ml of fluid in the test tube. The cells were thoroughly suspended and 3 ml of 0.56% KCl hypotonic solution was added and the suspension was incubated in a 37°C water bath for 20 min. The cells were again collected by centrifuging at 1000 rpm for 5 min. The supernatant was removed with a pasteur pipet leaving approximately 0.25 ml of fluid in which the cells were resuspended. Then 10 ml of fresh fixative (3 parts absolute methanol: 1 part glacial acetic acid) was slowly added with constant agitation to prevent cell clumping. Five aspirations with a pasteur pipet aided mixing. The fixative was changed two times. During the last resuspension approximately 4 ml fixative was added to give a turbid cell suspension.

Precleaned microscope slides previously placed in a freezer were allowed to collect condensation. Two to four drops of cell suspension were dropped on each slide from 12-18 in. by use of a pasteur pipet. The slides were immediately passed through a flame allowing the methanol to burn completely. Once completely dry, the slides were flooded for 4-5 min with fresh Giemsa stain prepared by mixing 5 ml stock solution, 6 ml acetone, and 100 ml tap water. The Giemsa stock solution contained 3.6 g Giemsa powder (Polysciences Inc.) dissolved in 250 ml glycerol and 250 ml methanol. After staining, the slides were rinsed in distilled water and were dried with a stream of air. Slides were blind scored for gaps, chromatid and chromosome breaks, and for pulverized metaphase spreads under 1000 magnification.

To determine possible c-mitotic effect of PBB, mitotic and metaphase indices were determined from animals treated as for chromosome analysis, but without colchicine injection. To detect possible c-mitosis synergism between PBB and colchicine, the slides used for chromosome analysis were also scored for mitotic and metaphase indices.

Statistical Analysis

The significance of differences of chromosome aberrations and metaphase and mitotic indices in treated and control groups was determined by the method of Kastenbaum and Bowman (7).

Table 1. Evaluation of control, PBB, or TEM-treated mouse bone marrow metaphases for chromosome abnormalities 12, 24, and 48 hr after treatment.

Treatment, mg/kg	Time hr	No. of animals	Total no. of cells scored	No. of cells		Cells with gaps, %	No. of cells					Cells with aberrations, %
				Centromeric gaps	Noncentromeric gaps		Chromatid break	Chromosome break	Multiple Chromatid break	Multiple chromosome breaks	Pulverized cells	
Control	12	3	300	3	2	3.33	2	0	0	0	0	0.66
PBB 50		3	300	12	1	4.33	1	0	0	1	0	0.66
PBB 500		3	300	14	0	4.66	0	1	0	0	0	0.33
TEM 4.5		3	300	6	10	5.33	5	2	16[b]	0	16[b]	13.00[b]
Control	24	3	300	11	0	3.66	0	0	0	0	0	0.00
PBB 50		3	300	20	8[a]	9.33[a]	4	0	0	0	0	1.33
PBB 500		3	300	15	2	5.66	1	0	0	0	0	0.33
TEM 4.5		3	300	41[b]	22[b]	21.00[b]	18[b]	0	15[b]	0	0	11.00[b]
Control	48	3	300	2	0	0.66	0	0	0	0	0	0.00
PBB 50		3	300	6	1	2.33	1	0	0	0	0	0.33
PBB 500		3	300	12[a]	1	4.33[a]	0	0	0	0	0	0.00
TEM 4.5		3	300	54[b]	8[a]	20.66[b]	8[a]	11[b]	1	3	1	8.00[b]

[a] $p < 0.05$; significant.
[b] $p < 0.01$; highly significant.

Table 2. Evaluation of control, PBB-, TEM-, or colchicine-treated bone marrow cells for mitotic and metaphase indices 12, 24, and 48 hr after treatment.

Treatment, mg/kg	Colchicine, mg/kg	No. of animals	12 hr		24 hr		48 hr	
			No. of cells counted	Cells in mitosis, %	No. of cells counted	Cells in mitosis, %	No. of cells counted	Cells in mitosis, %
				Mitotic Index				
Control	0	3	3035	0.5	3024	0.8	3009	1.4
Control	5	3	3031	4.6	3030	6.7	3021	8.3
PBB 50	0	3	3026	1.2	3010	1.3	3016	1.0
PBB 50	5	3	3027	4.8	3025	3.3	3103	7.0
PBB 500	0	3	3037	1.7	3018	1.4	3025	1.8
PBB 500	5	3	3038	5.7	3027	8.5	3016	6.7
TEM 4.5	0	3	3024	1.0	3030	0.3	3022	1.4
TEM 4.5	5	3	3026	3.7	3016	1.1	3102	4.3
				Metaphase Index				
Control	0	3	3035	0.0	3024	0.1	3009	0.3
Control	5	3	3031	1.1	3030	1.6	3021	1.6
PBB 50	0	3	3026	0.2	3010	0.2	3016	0.2
PBB 50	5	3	3027	1.2	3025	1.0	3103	2.1
PBB 500	0	3	3037	0.4	3018	0.1	3025	0.2
PBB 500	5	3	3038	1.6	3027	2.4	3016	1.7
TEM 4.5	0	3	3024	0.1	3030	0.0	3022	0.2
TEM 4.5	5	3	3026	0.9	3016	0.3	3102	1.9

Table 3. Evaluation of fetuses from polybrominated biphenyl-treated pregnant rats.

PBB, mg/kg[a]	No. of animals	No. of fetuses	Sex of fetus		No. of fetuses per animal	Average weight of fetuses, g	Dead implants, %
			Males, %	Females, %			
0	14	168	50.6	49.4	12.0	1.75	8.3
600	15	208	51.0	49.0	13.8	1.67	9.6

[a] PBB administered on day 6, 8, 10, 12, 14, and 16 of pregnancy in 100 mg/kg oral doses in 1 ml corn oil.

Results and Discussion

A highly significant increase of gaps was detected 24 and 48 hr after treatment with 4.5 mg/kg oral dose of TEM. After treatment with TEM, a highly significant increase of multiple chromatid breaks and cells with pulverized chromosomes, chromatid and multiple chromatid breaks, and chromosome breaks were detected 12, 24, and 48 hr, respectively. These results show that the system used will detect chromosome abnormalities if they are present. A dose of 50 or 500 mg PBB/kg failed to increase significantly chromosome or chromatid breaks, but 50 mg PBB/kg at 24 hr and 500 mg PBB/kg at 48 hr did increase the number of gaps significantly (Table 1). However the genetic significance of gaps is unclear (*8*, *9*).

Table 4. PBB–Colchicine synergism in pregnant rats for bone marrow mitotic and metaphase endices.

Treatment		No. of animals	Total no. of cells	Metaphase index, %	Miotic index, %
PBB, mg/kg[a]	Colchicine, mg/kg[b]				
0	0	5	9977	0.46	2.16
0	5	5	6939	1.91[c]	5.37[c]
600	0	6	17825	0.55	2.20
600	5	6	5886	4.79[d]	12.40[d]

[a] PBB administered on day 6, 8, 10, 12, 14, and 16 of pregnancy in 100 mg/kg oral doses in 1 ml corn oil.

[b] Colchicine administered on day 19 of pregnancy 5 hr prior to killing animals.

[c] Difference highly significant when compared to 0 mg/kg PBB, 0 mg/kg colchicine treatment.

[d] Difference highly significant when compared to 0 mg/kg PBB, 5 mg/kg colchicine treatment.

Mitotic index can serve as an indicator of the bioavailability of the test compound or its metabolites if a positive response occurs (5). PBB did not increase either mitotic or metaphase index in mice bone marrow (Table 2). Also, PBB did not show synergism with colchicine in mice as reported earlier in rats (6). We do not have an explanation for synergism of PBB and colchicine in rats or of its absence in mice. But it may be pertinent that PBB–colchicine synergism in female rats was detected after six oral doses of 100 mg/kg PBB suspended in corn oil administered in two day intervals. The animals were killed 72 hr after the last treatment for teratogenic and cytogenetic evaluations (6). (Tables 3 and 4). In contrast, male mice received single oral doses of 50 and 500 mg/kg PBB dissolved in DMSO, and the animals were killed 12, 24, and 48 hr after treatment for cytologic evaluations (Table 2).

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